

# SN

## 中华人民共和国出入境检验检疫行业标准

SN/T 4675.23—2016

### 出口葡萄酒及葡萄汁中氨氮的测定 连续流动分析仪法

Determination of ammonium nitrogen in wine and grape juice for export—  
Continuous flow analysis(CFA) method

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## 前 言

SN/T 4675《出口葡萄酒质量安全分析方法》共分为 30 个部分：

- SN/T 4675.1 出口葡萄酒中甘油的测定 酶法；
- SN/T 4675.2 出口葡萄酒中 2,3-丁二醇的测定 气相色谱法；
- SN/T 4675.3 出口葡萄酒中乙醇稳定碳同位素比值的测定；
- SN/T 4675.4 出口葡萄酒中乳酸的测定 酶法；
- SN/T 4675.5 出口葡萄酒中有机酸的测定 离子色谱法；
- SN/T 4675.6 出口葡萄酒中葡萄糖、果糖和蔗糖的测定；
- SN/T 4675.7 出口葡萄酒中乙醛的测定 气相色谱-质谱法；
- SN/T 4675.8 出口葡萄酒中 5-羟甲基糠醛的测定 液相色谱法；
- SN/T 4675.9 出口葡萄酒中二甘醇的测定 气相色谱-质谱法；
- SN/T 4675.10 出口葡萄酒中赭曲霉毒素 A 的测定 液相色谱-质谱/质谱法；
- SN/T 4675.11 出口葡萄酒中 7 种花色苷的测定 超高效液相色谱法；
- SN/T 4675.12 出口葡萄酒中溶菌酶的测定 液相色谱法；
- SN/T 4675.13 出口葡萄酒中 2,4,6-三氯甲苯醚残留量的测定 气相色谱-质谱法；
- SN/T 4675.14 出口葡萄酒中纳他霉素的测定 液相色谱-质谱/质谱法；
- SN/T 4675.15 出口葡萄酒中水杨酸、脱氢乙酸和对氯苯甲酸的测定 液相色谱法；
- SN/T 4675.16 出口葡萄酒中富马酸的测定 液相色谱-质谱/质谱法；
- SN/T 4675.17 出口葡萄酒中丁基锡含量的测定 气相色谱-质谱/质谱法；
- SN/T 4675.18 出口葡萄酒中二硫代氨基甲酸酯残留量的测定 顶空气相色谱法；
- SN/T 4675.19 出口葡萄酒中钠、镁、钾、钙、铬、锰、铁、铜、锌、砷、硒、银、镉、铅的测定；
- SN/T 4675.20 出口葡萄酒中稀土元素的测定 电感耦合等离子体质谱法；
- SN/T 4675.21 出口葡萄酒中可溶性无机盐的测定 离子色谱法；
- SN/T 4675.22 出口葡萄酒中总二氧化硫的测定 比色法；
- SN/T 4675.23 出口葡萄酒及葡萄汁中氨氮的测定 连续流动分析仪法；
- SN/T 4675.24 出口葡萄酒福林-肖卡指数的测定 分光光度计法；
- SN/T 4675.25 出口葡萄酒颜色的测定 CIE 1976(L\*a\*b\*)色空间法；
- SN/T 4675.26 出口葡萄酒浊度的测定 散射光法；
- SN/T 4675.27 出口葡萄酒碱性灰分的测定；
- SN/T 4675.28 出口葡萄酒细菌、霉菌及酵母的计数；
- SN/T 4675.29 出口葡萄酒中酒香酵母检验 实时荧光 PCR 法；
- SN/T 4675.30 出口葡萄酒中拜氏接合酵母检验 实时荧光 PCR 法。

本部分为 SN/T 4675 的第 23 部分。

本部分按照 GB/T 1.1—2009 给出的规则起草。

本部分由国家认证认可监督管理委员会提出并归口。

本部分起草单位：中华人民共和国北京出入境检验检疫局、中华人民共和国上海出入境检验检疫局、中华人民共和国广东出入境检验检疫局。

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## 出口葡萄酒及葡萄汁中氨氮的测定 连续流动分析仪法

### 1 范围

SN/T 4675 的本部分规定了出口葡萄酒及葡萄汁中氨氮含量的连续流动分析仪的测定方法。  
本部分适用于葡萄酒及葡萄汁中氨氮的测定。

### 2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件,仅注日期的版本适用于本文件。凡是不注日期的引用文件,其最新版本(包括所有的修改单)适用于本文件。

GB/T 6682 分析实验室用水规格和试验方法

### 3 方法提要

样品与试剂在蠕动泵的推动下进入化学反应模块,在密闭的管路中连续流动,被气泡按一定间隔规律地隔开。在碱性介质中,样品中的氨、铵离子与二氯异氰尿酸钠溶液释放出来的次氯酸根反应生成氯胺。在 40 ℃ 和亚硝基铁氰化钠存在的条件下,氯胺与水杨酸盐反应形成蓝绿色化合物,于 660 nm 波长处测量吸光度,外标法定量。

参考工作流程图,见图 1。

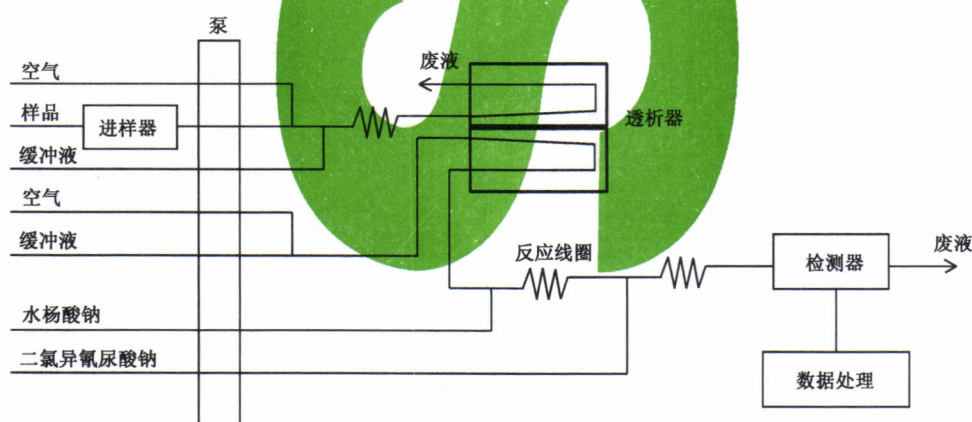


图 1 连续流动分析法测定葡萄酒及葡萄汁中氨氮含量流程图

### 4 试剂和材料

除非另有说明,所有试剂均为分析纯,水为 GB/T 6682 规定的二级水。

4.1 氯化铵( $\text{NH}_4\text{Cl}$ ):优级纯,在 105 ℃ $\pm$ 1 ℃下干燥恒重后,保存在干燥器中。

4.2 乙醇( $\text{C}_2\text{H}_5\text{OH}$ )。



- 4.3 氢氧化钠(NaOH)。
- 4.4 柠檬酸三钠( $C_6H_5O_7Na_3 \cdot 2H_2O$ )。
- 4.5 水杨酸钠( $NaC_7H_5O_3$ )。
- 4.6 二氯异氰脲酸钠( $C_3Cl_2N_3O_3Na \cdot 2H_2O$ )。
- 4.7 亚硝基铁氰化钠( $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ )。
- 4.8 十二烷基聚乙二醇醚(Brij35,  $C_{58}H_{118}O_{24}$ )。
- 4.9 乙醇溶液:12%乙醇溶液。量取 120 mL 乙醇(4.2),用水定容至 1 000 mL,混匀。
- 4.10 十二烷基聚乙二醇醚(Brij35)溶液:30% Brij35 溶液。称取 30.0 g Brij35(4.8)溶于 100 mL 水中,混匀。
- 4.11 氢氧化钠溶液:5 mol/L 氢氧化钠溶液。称取 200.0 g 氢氧化钠(4.3)溶于适量水中,冷却后,用水稀释至 1 000 mL,混匀。
- 4.12 缓冲溶液:称取 40.0 g 柠檬酸三钠(4.4),用水定容至 1 000 mL,加入 1 mL 30% Brij35(4.10),混匀。
- 4.13 水杨酸钠溶液:称取 34.0 g 水杨酸钠(4.5)及 0.4 g 亚硝基铁氰化钠(4.7),用水定容至 1 000 mL,加入 1 mL 30% Brij35(4.10),混匀。
- 4.14 二氯异氰脲酸钠溶液:称取 2.0 g 二氯异氰尿酸钠(4.6),加入氢氧化钠溶液(4.11)50 mL,加水定容至 1 000 mL,混匀。
- 4.15 氨氮标准储备液:准确称取 3.819 g 氯化铵(4.1)加水溶解并定容至 1 000 mL 容量瓶中,混匀。或直接购买市售有证标准溶液  $\rho(N)=1\ 000\ mg/L$ 。
- 4.16 氨氮标准中间溶液:酒精含量为 8%~15%葡萄酒样品的测定:10 mL 氨氮标准储备液(4.15)加入到 100 mL 容量瓶中,用 12%乙醇-水溶液定容至刻度,混匀。酒精含量高于 15%或低于 8%的葡萄酒样品的测定:10 mL 氨氮标准储备液(4.15)加入到 100 mL 容量瓶中,用与样品酒精度相同的乙醇-水溶液定容至刻度,混匀;葡萄汁样品的测定:10 mL 氨氮标准储备液(4.15)加入到 100 mL 容量瓶中,用水定容至刻度,混匀。
- 4.17 滤膜(水系):孔径为 0.45  $\mu m$ 。

## 5 仪器和设备

- 5.1 天平:精度为 0.1 g,0.1 mg。
- 5.2 实验仪器:连续流动分析仪,配有透析器。
- 5.3 容量瓶:100 mL,200 mL,1 000 mL。
- 5.4 移液器:100  $\mu L$ ~1 000  $\mu L$ ,500  $\mu L$ ~5 000  $\mu L$ 。

## 6 操作步骤

### 6.1 样品前处理

不带气泡样品经滤膜(4.17)过滤后直接进样;起泡葡萄酒需预先脱气。将 100 mL 试样倒入带排气塞的瓶中,在室温下使用水平振荡器或超声波水浴脱气,直至无气泡逸出。

### 6.2 测定步骤

#### 6.2.1 标准曲线的绘制

乙醇含量为 8%~15%的葡萄酒样品标准曲线的配制:量取适量的氨氮标准中间溶液(4.16),用



12%乙醇-水溶液稀释定容至 100 mL,制备 6 个浓度点的标准系列,氨氮浓度分别为:0 mg/L、10 mg/L、20 mg/L、30 mg/L、40 mg/L 和 60 mg/L。

乙醇含量低于 8%或高于 15%的葡萄酒样品标准曲线的配制:量取适量的氨氮标准中间溶液(4.16),用与检测样品标示的乙醇含量等同的乙醇-水溶液稀释氨氮标准中间溶液(4.16)并定容至 100 mL,制备 6 个浓度点的标准系列,氨氮浓度分别为:0 mg/L、10 mg/L、20 mg/L、30 mg/L、40 mg/L 和 60 mg/L。

葡萄汁样品标准曲线的配制:量取适量的氨氮标准中间溶液(4.16),用水稀释定容至 100 mL,制备 6 个浓度点的标准系列,氨氮浓度分别为:0 mg/L、10 mg/L、20 mg/L、30 mg/L、40 mg/L 和 60 mg/L。

手动进样标准曲线最高浓度,待出峰后设置自动增益调节(一般为 90%)。再按编排好的程序开始运行,包括标准曲线、基线校正、带过校正、漂移校正等,软件会按峰高和浓度值自动绘制标准曲线。

### 6.2.2 测定

按照与绘制标准曲线相同的条件,量取适量样品进行测定。若样品的氨氮含量超出标准曲线检测范围,应取适量样品稀释后上机测定。

### 6.2.3 空白试验

用实验用水代替样品,按照 6.2.2 步骤进行空白试验。

## 7 结果计算与表示

7.1 样品中氨氮的含量(以氮计,mg/L),按照式(1)进行计算。

$$x = (c - c_0) \times f \quad \dots\dots\dots (1)$$

式中:

$x$  ——样品中氨氮的含量,单位为毫克每升(mg/L);

$c$  ——由标准曲线得到的样液中氨氮的浓度,单位为毫克每升(mg/L);

$c_0$  ——由标准曲线得到的空白试验中氨氮的浓度,单位为毫克每升(mg/L);

$f$  ——稀释倍数。

7.2 结果保留 3 位有效数字。

## 8 定量限

葡萄汁和葡萄酒的定量限为 1 mg/L。

## 9 精密度

在重复性条件下获得两次独立测定结果差值的绝对值不得超过算术平均数的 5%。

## 10 回收率

葡萄酒、葡萄汁基质中的添加水平及回收率数据见表 1。

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表 1 不同浓度添加水平的添加回收率( $n=6$ )

样品	添加水平/(mg/L)	回收率范围/%	相对标准偏差/%
红葡萄酒	2	90.8~97.7	1.5
	5	93.0~97.1	3.4
	10	95.9~99.8	1.4
白葡萄酒	2	97.5~98.3	0.8
	5	96.3~99.1	2.0
	10	96.1~99.1	1.3
起泡葡萄酒	5	90.2~97.8	2.7
	10	92.6~98.0	2.4
	20	93.6~95.3	1.2
葡萄汁	100	96.3~96.7	0.5
	200	96.2~97.4	0.3
	400	99.2~99.5	0.2

## Foreword

Standard(SN/T 4675)“Methods of export wine analysis”includes 30 parts:

- SN/T 4675.1 Determination of glycerol in wine for export—Enzymatic method;
- SN/T 4675.2 Determination of 2,3-butanediol in wine for export—GC method;
- SN/T 4675.3 Determination of stable carbon isotope ratio of ethanol in wine for export;
- SN/T 4675.4 Determination of lactic acid in wine for export—Enzymatic method;
- SN/T 4675.5 Determination of organic acid in wine for export—Ion chromatography method;
- SN/T 4675.6 Determination of glucose, fructose and sucrose in wine for export;
- SN/T 4675.7 Determination of acetaldehyde in wine for export—GC/MS method;
- SN/T 4675.8 Determination of 5-hydroxymethylfurfural in wine for export—HPLC method;
- SN/T 4675.9 Determination of diethylene in wine for export—GC/MS method;
- SN/T 4675.10 Determination of ochratoxin A in wine for export—HPLC/MS/MS method;
- SN/T 4675.11 Determination of 7 anthocyanins in wine for export—UHPLC method;
- SN/T 4675.12 Determination of lysozyme in wine for export—HPLC method;
- SN/T 4675.13 Determination of 2,4,6-trichloroanazole in wine for export;
- SN/T 4675.14 Determination of natamycin in wine for export—HPLC/MS/MS method;
- SN/T 4675.15 Determination of salicylic acid, dehydroacetic acid and 4-chlorobenzoic acid in wine for export—HPLC method;
- SN/T 4675.16 Determination of fumaric acid in wine for export—HPLC/MS/MS method;
- SN/T 4675.17 Determination of butyltin compounds in wine for export—GC/MS/MS method;
- SN/T 4675.18 Determination of dithiocarbamates(salt) residues in wine for export—Headspace



SN/T 4675.23—2016

GC method;

—SN/T 4675.19 Determination of sodium, magnesium, potassium, calcium, chromium, manganese, iron, copper, zinc, arsenic, selenium, silver, cadmium and lead in wine for export;

—SN/T 4675.20 Determination of rare-earth elements in wine for export—ICP-MS method;

—SN/T 4675.21 Determination of soluble inorganic salts in wine for export—Ion chromatography method;

—SN/T 4675.22 Determination of total sulfur dioxide in wine for export—Colorimetric method;

—SN/T 4675.23 Determination of ammonium nitrogen in wine and grape juice for export—Continuous flow analysis(CFA) method;

—SN/T 4675.24 Determination of Folin & Ciocalteu index of wine for export—Spectrophotometry method;

—SN/T 4675.25 Determination of chromatic characteristics of wine for export—CIE Lab color space system;

—SN/T 4675.26 Determination of turbidity of wine for export—Diffused radiation method;

—SN/T 4675.27 Determination of alkaline ash of wine for export;

—SN/T 4675.28 Method for enumeration of colony-forming units of yeasts, moulds and bacteria in cork stoppers and wine for export;

—SN/T 4675.29 Determination of brettanomyces in wine for export—Real-time PCR method;

—SN/T 4675.30 Determination of zygosaccharomyces bailii in wine for export—Real-time PCR method.

This part is part 23 of the standard.

This part is drafted according to GB/T 1.1—2009.

Please note that some of the content of the standard may involve patents. Publication of the present standard does not bear the responsibility of identifying these patents.

This part was proposed by and was under the jurisdiction of Certification and Accreditation Administration of the People's Republic of China.

This part was drafted by Beijing Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Guangdong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this part were Liu Ying, Han Shen, Cheng jia, Liu Xiyan, Wang Peiyue, Cheng Chao, Liu Qing, Li Zhiyong.

# Determination of ammonium nitrogen in wine and grape juice for export— Continuous flow analysis(CFA) method

## 1 Scope

The standard specifies methods suitable for the determination of ammonium nitrogen in wine and grape juice for export, applying for continuous flow analysis (CFA).

This standard is applicable to the determination and quantification of ammonium nitrogen in red wine, white wine, pink wine, sparkling wine, ice wine and grape juice for export by continuous flow analysis (CFA).

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For date-references, only edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682 water for analytical laboratory use—Specification and test methods

## 3 Principle

In a continuously flowing, gas-segmented carrier stream, ammonium present in the sample reacts in alkaline solution with hypochlorite ( $\text{ClO}^-$ ), which has previously been liberated from dichloroisocyanurate. The chloroamine formed reacts under catalysis of nitroprusside with salicylate at  $40\text{ }^\circ\text{C}$  to form a blue-green indophenol dye which is quantitatively measured in a flow photometer at  $660\text{ nm}$  by external standard method.

Flow analysis system is showed in figure 1.



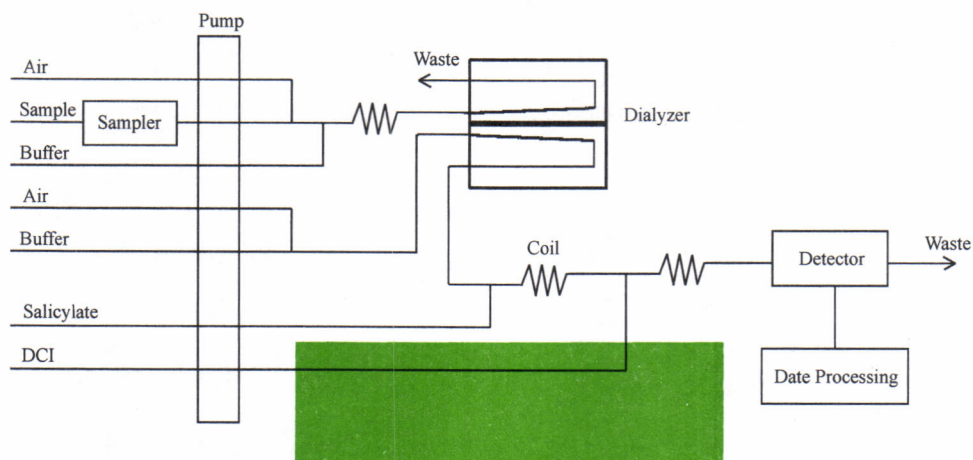


Figure 1—Flow analysis system for determination ammonium in wine and grape juice

#### 4 Reagents and materials

Unless otherwise specified, all reagents should be of analytical grade. Water is the second grade water prescribed by GB/T 6682.

4.1 Ammonium chloride:  $\text{NH}_4\text{Cl}$ ; dried at  $105\text{ }^\circ\text{C} \pm 2\text{ }^\circ\text{C}$  to constant mass.

4.2 Ethanol:  $\text{C}_2\text{H}_5\text{OH}$ .

4.3 Sodium hydroxide:  $\text{NaOH}$ .

4.4 Trisodium citrate dehydrate:  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ .

4.5 Sodium salicylate:  $\text{NaC}_7\text{H}_5\text{O}_3$ .

4.6 Sodium dichloroisocyanurate dehydrate (sodium 1,3-dichlorohexahydro-1,3,5-triazine-2,4,6-trione):  $\text{NaC}_3\text{Cl}_2\text{N}_3\text{O}_3 \cdot 2\text{H}_2\text{O}$ .

4.7 Sodium nitroprusside dihydrate[sodium nitrosopentacyanoferrate(II)]:  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$ .

4.8 Polyethylene glycol dodecyl ether(Brij35,  $\text{C}_{58}\text{H}_{118}\text{O}_{24}$ ).

4.9 12% (V/V) ethanol: 120 mL of ethanol(4.2) diluted to 1 000 mL.

4.10 30% Brij35: dissolve 30.0 g of Brij35(4.8) in 100 mL water.

4.11 Sodium hydroxide solution: dissolve 200.0 g of sodium hydroxide (4.3) in water, and make up to 1 000 mL with water.

4.12 Buffer solution: in a 1 000 mL graduated flask, dissolve 40.0 g of trisodium citrate dehydrate (4.4) and 1 mL of 30% Brij35(4.10) in water and make up to volume with water.

4.13 Sodium salicylate solution: in a 1 000 mL graduated flask, dissolve 34.0 g of sodium salicylate (4.5), 0.4 g of sodium nitroprusside dehydrate (4.7) and 1 mL 30% Brij35(4.10) with water, and make up to volume with water.

4.14 Sodium dichloroisocyanurate dehydrate solution: in a 1 000 mL graduated flask, dissolve 2.0 g sodium dichloroisocyanurate dehydrate (4.6) and 50 mL sodium hydroxide solution(4.11) with water and make up to volume with water.

4.15 Ammonium stock solution:  $\rho(\text{N}) = 1\,000\text{ mg/L}$ , in a 1 000 mL graduated flask, dissolve 3.819 g ammonium chloride (4.1) in water and make up to volume. Or, buy directly the standard solution with certificate.

4.16 Ammonium standard solution: For wine with 8% ~ 15% ethanol content: add 10 mL of stock solution (4.15) to about 80 mL of 12% ethanol. Dilute to 100 mL and mix thoroughly. For wine with ethanol content out of range of 8% ~ 15%: Dilute the stock solution (4.15) with the ethanol solution same to the wine. Dilute to 100 mL and mix thoroughly. For grape juice, add 10 mL of stock solution (4.15) to about 80 mL of water. Dilute to 100 mL and mix thoroughly.

4.17 Membrane filter: 0.45  $\mu\text{m}$ , hydrophilic.

## 5 Apparatus

5.1 Analytical balance: sensitivity at 0.1 mg.

5.2 Equipment: Flow injection system with dialysis.

5.3 Graduated flasks: 100 mL, 200 mL and 1 000 mL.

5.4 Graduated pipettes: 100  $\mu\text{L}$  ~ 1 000  $\mu\text{L}$ , 500  $\mu\text{L}$  ~ 5 000  $\mu\text{L}$ .

## 6 Procedure

### 6.1 Sample pretreatment

Analyze the sample directly after filtering with the membrane (4.17). Sparkling wine need to be degassed Pour the wine sample of about 100 mL into a beaker flask with air evacuation valve. Placed the wine in an ultrasonic water tank( or horizontal oscillator) at room temperature after a certain period of time until no gas escapes.

### 6.2 Determination

#### 6.2.1 Calibration solutions

Calibration solution of wine within the range of 8% ~15% ethanol: Pipette the suitable ammonium standard solution (4.16) respectively into 100 mL graduated flask and make up to volume with 12% ethanol. The mass concentrations of ammonium, expressed as nitrogen, in these calibration solutions are 0 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 60 mg/L.

Calibration solution of wine beyond the range of 8% ~15% ethanol: Pipette the suitable ammonium standard solution (4.16) respectively into 100 mL graduated flask and make up to volume with the ethanol-water solution of the concentration of the wine. The mass concentrations of ammonium, expressed as nitrogen, in these calibration solutions are 0 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 60 mg/L.

Calibration solution of grape juice: Pipette the suitable ammonium stock solution (4.16) respectively into 100 mL graduated flask and make up to volume with water. The mass concentrations of ammonium, expressed as nitrogen, in these calibration solutions are 0 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 60 mg/L.

Manual inject the highest concentration of the work solution, set the automat gain adjustment (normally 90%) after presenting the peak. Then run the procedure which was set in advance including standard curve, baseline correction, band over correction, drift correction and etc. The software could draw the standard curve according to the relationship between the height of peak and mass concentrations.

#### 6.2.2 Measurement

Analyze the samples, in the same way as the calibration solutions with the continuous flow system. If the ammonium concentration is beyond the range of the calibration curve, suitable sample should be diluted and injected again.



### 6.2.3 Reagent blank check

Instead of reagent solutions, run water through the system as 6.2.2.

## 7 Calculation

7.1 Determine the mass concentration of ammonium (expressed as nitrogen) in the measuring solution using the measured value obtained as described in 6.2.2 from the function (1) as follows.

$$x = (c - c_0) \times f \quad \dots\dots\dots (1)$$

Where:

$x$  —the mass concentration of ammonium in the sample, mg/L;

$c$  —the concentration of ammonium in the solution corresponding to the standard working solution, mg/L;

$c_0$  —the concentration of ammonium in the reagent blank corresponding to the standard working solution, mg/L;

$f$  —dilution factor.

7.2 Report results to three significant figures.

## 8 Limit of quantification

The limit of quantification of wine and grape juice is 1 mg/L.

## 9 Precision

The absolute value of the difference of two independent measurements under the same condition could not be more than 5% of the arithmetic mean.

## 10 Recovery

The spiked levels, recovery and RSD of ammonium nitrogen in red wine, rose wine and sparkling

wine are showed in Table 1.

Table 1—The recovery under different spiked concentrations( $n=6$ )

sample	Spiked level mg/L	Recovery range %	RSD %
Red wine	2	90.8~97.7	1.5
	5	93.0~97.1	3.4
	10	95.9~99.8	1.4
White wine	2	97.5~98.3	0.8
	5	96.3~99.1	2.0
	10	96.1~99.1	1.3
Sparkling wine	5	90.2~97.8	2.7
	10	92.6~98.0	2.4
	20	93.6~95.3	1.2
Grape juice	100	96.3~96.7	0.5
	200	96.2~97.4	0.3
	400	99.2~99.5	0.2



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